Studies on Cell Inactivation Efficiency of Gamma and Proton Radiation Using MTT Assay

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Abstract: *The radiation damage of proton beam on tissues and cells has attracted attention and the unique radiobiological effects make proton radiation a useful tool for the investigation of radiation damage mechanisms. Potential use of protons and other charged particles for cancer treatment offer several advantages over low LET radiation cancer therapy. In the present study the effect of different doses of gamma and proton beams from the Folded Tandem Ion Accelerator (FOTIA) on human tumor adeno carcinoma (HT 29) cells has been analysed using MTT (Thiazolyl Blue Tetrazolium Bromide) assay. The cell proliferation of HT 29 cells after irradiation has been anlysed using MTT assay after a post irradiation incubation of 72 hrs. The results of these investigations are presented and discussed in the paper.*

Key words: *Proton beam, gamma radiation, accelerator, cell survival, MTT assay*

Introduction

Radiation plays an important role in life science and medicine. Much of radiobiology is done with photons but the behaviour of charged particles such as protons and heavy ions are different and they can be confined by magnetic and electric field and thus be accelerated and directed as needed [Hartmut F. W Sadrozinski, 2003]. The goal of radiotherapy is to maximize tumor-cell killing and to minimize normal tissue damage. Each normal and malignant tissue has its own radiobiological properties and biological parameters, and the response of normal and tumor tissues depends on the temporal pattern of radiation delivery such as dose, dose rate and dose fractionation and the presence of radiation sensitizers and protectors. At present, use of electron and proton radiation is constantly gaining importance in therapy applications. It offers many advantages over conventional ^{60}Co teletherapy, such as better dose profile and drastic reduction in dose to the normal tissues beyond the tumor. A clear

understanding of the biophysical aspects at different irradiation conditions has relevance in assessment of damage in biological systems and in radiation protection.

The present investigation was envisaged to compile and analyze the radiobiological effect of different radiation such as ^{60}Co gamma rays, and 6 MV proton beam. Gamma and proton irradiation studies have been carried out on human tumour adeno carcinoma (HT29) cells and the Thiazolyl blue Tetrazolium Bromide (MTT) Assay has been carried out to find the cell survival.

Materials and Methods

Radiation Sources

Gamma Chamber - 900

Gamma Chamber - 900 is a compact self-shielded ^{60}Co gamma irradiator providing an irradiation volume of ~ 900 cc. The source pencils are placed concentrically in a cylindrical geometry. The samples for irradiation are placed in an irradiation chamber, which is cylindrical in geometry. This shaft can be moved up and down with the help of a system of motorized drive, which enables precise positioning of the irradiation chamber at the centre of the radiation field. Since samples were exposed from all sides, it gives better uniformity in dose distribution within the samples, as it reduces the depth dose and build up effects. A Perspex sample holder with 8 sample positions was used to deliver the precise dose. Using upward and downward switches, the shaft can be moved vertically downward for irradiation and upward to remove the irradiated samples.

The Folded Tandem Ion Accelerator

Proton beams are accelerated using the FOTIA facility at Bhabha Atomic Research Centre (BARC) (Singh P 2001). The FOTIA is an electrostatic accelerator with a maximum terminal voltage of 6 MV. In FOTIA, the negative ion beams extracted from the SNICS-II source are pre-accelerated up to 150 keV. Negative ions of the desired mass are selected using a 70^0 dipole magnet and injected into the low energy accelerating tube through a 20^0 electrostatic deflector. An electrostatic quadrupole triplet is used to focus and match the beam parameters to the acceptance of the low energy tube. The electrons of these accelerated negative ions get stripped off at the stripper and the charge state of the positive ions thus produced is selected with the 180^0 magnet inside the high voltage

terminal before being bent into the high energy accelerating tube where they further accelerated. At the exit of the 180^0 magnet, the beam diverges. An electrostatic quadrupole doublet is used to focus the beam before it enters the high energy tube. The beams accelerated in the high energy accelerating tube are focused using a magnetic quadrupole triplet. The beam is transported to the experimental area using a magnetic quadrupole triplet and a switching magnet. Beam profile monitors and Faraday cups have been installed in the beam line to measure the size, shape and position of the beam.

The beam was characterized by performing the Rutherford Back Scattering (RBS) on gold, tin and iron targets which were in the form of self-supporting foils. The targets were mounted inside the 80 cm diameter scattering chamber installed in the beam line direction. A thin target of ^{197}Au was detected using the surface barrier detector mounted at an angle of 80^0 on one of the arms in the scattering chamber. To calibrate the pulse height of the detector, an alpha source was kept on one of the target holders. The details are given elsewhere (Singh P, 2001).

The primary proton beam from the FOTIA was collimated using an adjustable slit to reduce the fluence and then diffused using a gold foil. The diffused beam was channeled to the exit window made of 20 μm titanium foil of 3 cm diameter to get uniformly distributed irradiation area. The average beam current was 2 nA with a beam size of about 3X 3 cm on the sample. Both the fluence and beam energy were measured at the sample position using a SSB detector. Another SSB detector placed inside the scattering chamber at a forward angle of 80° to the primary beam, after the gold foil, served as a monitor detector. The ratio of the monitor detector counts to that of the flux measured using SSB detector at the sample position was measured by multiple trials and the calibration factor was obtained. Monitor detector counts and the measured ratio were used for delivering the required fluence to the samples. The beam profile at the sample position was also measured using SSB detector. The samples were thus irradiated under normal atmospheric pressure at 24^0C. The target to be irradiated was positioned at a distance of 11 mm from the exit window, that being the closest possible place to mount the target.

Sample Preparation for Irradiation

HT 29 is a cell line derived from human colorectal cancer cells and it can be used for tumorogenic studies. For the present studies HT 29

cells were obtained from National Centre for Cell Sciences (NCCS), Pune, India.

HT 29 cells were grown in 25 ml cell culture dishes (Falcon) and maintained in Dulbecco's modified essential medium (DMEM) supplemented with 10% fetal calf serum (FCS; Himedia) at 37^0C in a humidified atmosphere containing 5% CO_2. Cells were passaged every 7 days at a 1:10 split and the experiments were performed on the day of 70-80% confluence. Cultured cells were harvested with 0.025% trypsin EDTA and washed in complete media by centrifugation and diluted to a concentration of 2 X 10^5 cells ml^{-1}. The cells attached to 3 cm diameter petri-plates were mounted vertically at the beam exit window for the proton irradiation.

Thiazolyl blue Tetrazolium Bromide (MTT) Assay

MTT assay offers a quantitative, convenient method for evaluating cell survival response to ionizing radiation. It is a colorimetric assay for measuring the activity of enzymes that reduce MTT to purple color formazan product. The amount of color produced is directly proportional to the number of viable cells. Yellow MTT (3 - (4, 5 - Dimethylthiazol-2-yl)-2, 5- diphenyltetrazolium bromide, a tetrazole) is reduced to purple formazan in living cells (Mosmann 1983). A solubilising solution such as DMSO is added to dissolve the insoluble purple formazan product into a colored solution (Altman 1976). Solubilization of the cells results in the liberation of the purple product which can be detected using a calorimetric measurement. The resulting purple solution is spectrophotometrically measured (Denizot and Lang 1986). An increase in cell number results in an increase in the amount of MTT formazan formed and an increase in absorbance. The ability of cells to reduce MTT provides an indication of the mitochondrial integrity and activity which, in turn, may be interpreted as a measure of the cell survival. When the amount of purple formazan produced by cells treated with radiation is compared with that produced by unirradiated control cells, the effectiveness of the agent in causing death of cells can be deduced, through the production of a dose-response curve. MTT assay has been carried out to understand the cell survival in HT 29 cells after proton and gamma irradiation.

After radiation treatment 100ìl of cell suspension in complete media was inoculated to each well of 96-well plates at the density of 2×10^4 cells/well (the area of each well was 0.32 cm^2). After 24 h of culture, 100ìl of media was added to each well. The total incubation time will be

depending on the doubling time of the cells used. After post irradiation incubation, the media was aspirated out of each well and 100µl of MTT solution prepared by dissolving the MTT dye (Thiazolyl blue tetrazolium bromide) in PBS to a concentration of 1 mg ml^{-1} was added and the plates were incubated in the dark for 4 h at 37°C. After incubation the dye was removed by inverting and tapping the plates and the formazan crystals formed were solubilized by incubating the cells with 200µl DMSO for 20 min at 37^0C in the dark. Absorbance at 550 nm wavelength was measured on a multi-well scanning spectrophotometer (EL_X 800) and the results were expressed as a percentage (%) of the control.

Statistical Analysis

Statistical analysis of the data was carried out using the software Microcal Origin Version 8. The differences in cell survival between control and irradiated groups and between two irradiated groups were anlysed using Student's *t*- test. Differences where $P < 0.05$ were considered to be statistically significant [Chaubey et al., 2001]. Data are presented as the mean ± standard deviation (S.D.)

Results and Discussions

Radiation plays an important role in life science and medicine. Much of radiobiology is done with photons but the behavior of charged particles such as protons and heavy ions are different and they can be confined by magnetic and electric field and thus be accelerated and directed as needed (Hartmut Sadrozinski 2003). In recent years the low energy heavy ion accelerators have an important role both in basic and applied sciences (Singh 2001). Potential use of protons and other charged particles in the cancer treatment offer several advantages over low LET radiation cancer therapy. An important feature of proton beams arises from the physical aspects of their dose distribution. Proton beams can provide highly localized, uniform doses of radiation to tumors, while sparing the surrounding normal tissues, compared with conventional modalities using photons or electrons (Sang Soo Kim *et al*., 2011). Although there are no randomized clinical trials comparing proton beam therapy with conventional X-ray therapy, the superiority of clinical effectiveness of proton beam therapy might result from its previously mentioned physical characteristics (Goitein and Cox 2008). This is why proton beam therapy is considered as a promising new treatment for malignant tumors.

The cells were exposed to different doses of radiation under normal atmospheric pressure at 24^0C. The total dose delivered to the sample

varied from 0 to 15 and the dose rate used was 2 Gy min^{1}. Immediately after irradiation, 100ìl of cell suspension in the media was inoculated into each well of 96-well plates (Falcon) at a density of 2×10^{4} cells per well and the cells were incubated for 72 hrs. After the incubation MTT assay has been carried out to understand the cell survival after exposure with different doses of proton and gamma radiation. The iso-effective doses obtained from the fitted curves were compared between the proton beam and reference gamma rays.

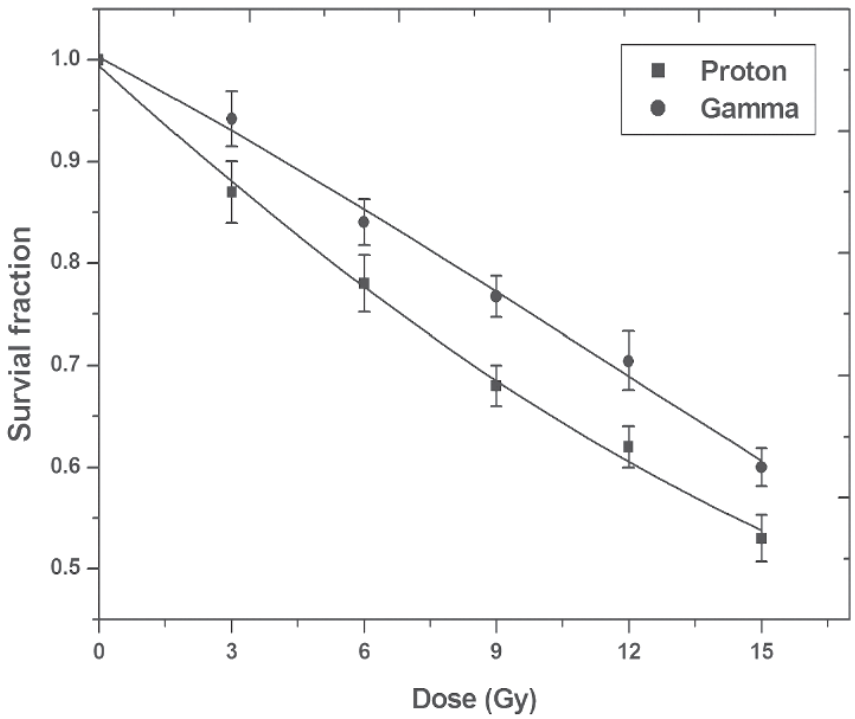

Figure 1: Cell proliferation of HT 29 cells with dose after irradiation with proton and gamma radiation measured using MTT assay.

Dose response curves for HT 29 cells exposed to different doses of 3.2 MeV protons and gamma radiation are shown in figure 1. The samples were irradiated with different doses ranging from 0 to 15 Gy. MTT assay was applied to quantify the cell proliferation after radiation treatment.

In the figure 6.1, the dose – cell survival relationship has been represented by a Sigmoidal (dose response) fit with $\div^2$ = 2.33E-4 for proton and 2.96E-4 for gamma radiation. A statistically significant decrease in cell survival with increase in dose is observed. From the figure it is clear that for the proton beam cell proliferation decreases with the number of ions traversing the cellular nuclei and the cell inactivation efficiency is more for proton in comparison with gamma radiation.

Conclusion

Dose survival response of HT 29 cells exposed to different doses of 3.2 MeV protons from the FOTIA and gamma radiation from ^{60}Co source has been studied using MTT assay. A statistically significant decrease in cell survival with increase in dose was observed. The cell proliferation decreases with the number of proton particles traversing the cellular nuclei and the cell inactivation efficiency is more for proton in comparison with gamma radiation. Proton beam therapy can deliver high doses to the target while sparing surrounding healthy tissues compared with conventional X-ray therapy. The study has relevance in radiation therapy.

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